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Use of the Microscreen Phage-Induction Assay to Assess the Genotoxicity of 14 Hazardous Industrial Wastes

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16. ABSTRACT

The Microscreen phage-induction assay, which quantitatively measures the induction of prophage λ in Escherichia coli $WP2_s(\lambda)$, was used to test 14 crude (unfractionated) hazardous industrial waste samples for genotoxic activity in the presence and absence of metabolic activation. Eleven of the 14 wastes induced prophage, and induction was observed at concentrations as low as 0.4 picograms per ml. Comparisons between the mutagenicity of these waste samples in Salmonella and their ability to induce prophage λ indicate that the Microscreen phage-induction assay detected genotoxic activity in all but one of the wastes that were mutagenic in Salmonella. Moreover, the Microscreen assay detected as genotoxic 5 additional wastes that were not detected in the Salmonella assay. Partial chemical characterizations of the wastes showed high concentrations of carcinogenic metals, solvents, and chlorinated compounds, most of which are detected poorly by the Salmonella assay. However, recent studies of the induction of prophage by these chemical classes have suggested that phage induction may be a sensitive endpoint for these groups of chemicals. This may explain the enhanced ability of the Microscreen phage-induction assay to detect genotoxic activity in 5 additional wastes compared to the Salmonella assay. The applicability of the Microscreen phage-induction assay for screening hazardous wastes for genotoxic activity is discussed along with some of the problems associated with screening highly toxic wastes containing toxic volatile compounds.

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Use of the Microscreen Phage-Induction Assay to Assess the Genotoxicity of 14 Hazardous Industrial Wastes

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The Microscreen phage-induction assay, which quantitatively measures the induction of prophage λ in *Escherichia coli* $WP2_s(\lambda)$, was used to test 14 crude (unfractionated) hazardous industrial waste samples for genotoxic activity in the presence and absence of metabolic activation. Eleven of the 14 wastes induced prophage, and induction was observed at concentrations as low as 0.4 pg per ml. Comparisons between the ability of these waste samples to induce prophage and their mutagenicity in the *Salmonella* reverse mutation assay indicate that the phage-induction assay detected genotoxic activity in all but one of the wastes that were mutagenic in *Salmonella*. Moreover, the Microscreen assay detected as genotoxic five additional wastes that were not detected in the *Salmonella* assay. The applicability of the Microscreen phage-induction assay for screening hazardous wastes for genotoxic activity is discussed, as are some of the problems associated with screening highly toxic wastes containing toxic volatile compounds.

Key words: hazardous wastes, Microscreen assay, prophage induction, complex mixtures

INTRODUCTION

According to estimates by the U.S. Environmental Protection Agency (US EPA), more than 260 million metric tons of hazardous wastes are generated annually in the United States [Dietz et al, 1984]. Wastes are classified as hazardous if they possess one of a number of hazardous physical characteristics (eg, ignitability) or if they comprise certain industrial process wastes or contain specifically regulated chemicals [Federal Register, 1980]. However, the EPA's current definition of hazard-

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ous waste omits biological testing protocols to determine the potential health effects of the wastes.

The deficiencies of current regulations have led EPA to consider adding biological endpoints, including mutagenicity and carcinogenicity, to their definition of hazardous waste [Federal Register, 1983, 1984a,b]. In contrast to physical and chemical analyses, the identification of potential biological effects could provide more relevant information regarding risks to human health. The presence of known mutagens and carcinogens in many wastes affirms the importance of genetic toxicity testing in hazard evaluations of wastes.

The *Salmonella* reverse mutation assay developed by Dr. Bruce Ames and coworkers [Ames et al, 1975] has been used more than any other short-term test for determining the genotoxicity of hazardous wastes. Wastes and effluents tested in the *Salmonella* assay include municipal wastewaters and sludges [Clevenger et al, 1983; Somani et al, 1980], industrial process streams and discharges [Houk and Claxton, 1986; Andon et al, 1985; DeMarini et al, 1987a,b], effluents from energy-related technologies [Manabe et al, 1984; Epler et al, 1978], and effluents from pulp and paper mills (Douglas et al, 1983; Møller et al, 1986]. However, hazardous wastes can contain high concentrations of carcinogenic metals, chlorinated compounds, and solvents [US EPA, 1984] that are detected poorly by the *Salmonella* assay [Kier et al, 1986; Zeiger and Tennant, 1986; Zeiger, 1987; Claxton et al, 1987]. Thus, the genotoxic potential of some wastes may escape detection when evaluated by the *Salmonella* assay.

The Microscreen phage-induction assay developed by Rossman et al [1984] has been shown to detect some carcinogenic metals [Rossman et al, 1984], chlorinated pesticides [Houk and DeMarini, 1987], and solvents [Houk and DeMarini, in prep.] that are not mutagenic in *Salmonella*. In addition, it has been used to detect genotoxic activity in complex mixtures [Rossman et al, 1985]. It is a rapid and inexpensive assay that quantitatively measures the induction of prophage λ in *Escherichia coli* $WP2_s(\lambda)$. The induction of prophage is one of several events that may occur upon induction of the SOS response, which is a cellular reaction to DNA damage. Other manifestations of the SOS response include DNA repair and mutagenesis, and many agents that induce the SOS response in bacteria are also genotoxic in a variety of other organisms [D'Ari, 1985].

In the present study, we have used the Microscreen phage-induction assay to evaluate the genotoxicity of 14 crude hazardous waste samples from 1) several specific industrial sources, including petrochemical, pharmaceutical, and plastics manufacturers, and 2) composites of wastes from a variety of different manufacturing processes. The results are compared to those obtained in *Salmonella* and are discussed in terms of the chemical analysis available for these wastes. We also describe some of the problems associated with screening highly toxic wastes containing toxic volatile compounds, and we discuss the suitability of the Microscreen phage-induction assay for screening hazardous wastes for genotoxic activity.

MATERIALS AND METHODS

Bacterial Strains

The bacterial strains are derivatives of *E. coli* B/r. $WP2_s(\lambda)$ is a lambda lysogen of $WP2_s$ (*trpE*, *uvrA*); SR714 (*trpE*, $uvrD_3$) is the indicator strain. Both strains were

kindly furnished by Dr. Toby Rossman, Department of Environmental Medicine, New York University, Tuxedo, NY. Stationary-phase cultures of SR714 and log-phase cultures of $WP2_s(\lambda)$ were prepared as described previously [Houk and DeMarini, 1987]. Free phage were eliminated from the lysogenic culture by resuspending the cells in supplemented minimal medium (Vogel-Bonner minimal medium supplemented with 0.2% glucose and 20 μg/ml tryptophan) to a final concentration of $\sim 2 \times 10^7$ cells/ml.

Hazardous Waste Samples

Fourteen samples of hazardous industrial wastes (labeled A–O in Table I) were obtained from Edward L. Katz, Hazardous Waste Engineering Laboratory, US EPA, Cincinnati. Three of the waste samples were from petrochemical, pharmaceutical, and plastics manufacturers (A, B, and C, respectively). The remaining wastes (D–O) were composites of wastes from a variety of industrial sources that were collected by four commercial hazardous waste incineration facilities and classified according to their aqueous or organic properties.

Table I provides a cursory physical description of the wastes, all of which were liquids or semisolids. Some wastes were biphasic (settled sludge and liquid); others were slurries or liquids with suspended oil droplets. Wastes were mixed carefully by swirling prior to testing. Waste G, however, consisted of a thick sludge that could not be mixed with its liquid component; thus, only the liquid portion of this waste was tested.

Table II shows the results of the partial chemical analysis performed on the waste samples [US EPA, 1984]. These characterizations should not be viewed as indicative of the overall chemical composition of the wastes because the analysis was limited to specific organics and/or metals identified in the EPA Appendix VIII list of priority pollutants [US EPA, 1984]. In addition, for purposes of a previous study on the performance of hazardous waste incinerators [US EPA, 1984], seven of the waste samples (B, F, G, J, K, L, and M) were spiked with carbon tetrachloride and trichloroethylene. Thus, the concentrations of these two chemicals in these seven waste samples reflect this addition.

TABLE I. Physical Descriptions of Hazardous Wastes

Waste	Physical state	Description
A	Liquid	Black, very thin oil from a petrochemical manufacturing plant
B	Liquid	Black, oily liquid from a pharmaceutical manufacturer
C	Tar	Black, pourable tar produced during the production of plastics
D	Liquid	Composite of aqueous wastes; watery liquid with red oil droplets
F and G	Semisolid	Composite of organic wastes; biphasic gray sludge with reddish-brown liquid
H and I	Suspension	Composite of aqueous wastes; thin, gray slurry
J	Liquid	Composite of organic wastes; gray, thick liquid with suspended solids
K	Liquid	Similar to J, but lighter in color and thinner
L and M	Tar	Composite of organic wastes; black, thin, pourable tar
N and O	Liquid	Composite of aqueous wastes; clear, watery liquid

TABLE II. Concentration of Chemicals and Metals Identified in Hazardous Wastes (μg/g)

				Hazardous waste incineration facilities									
				1	2				3		4			
	Petro-Chemical	Pharma-ceutical	Plastics:	Aqueous	Organic		Aqueous		Organic		Organic		Aqueous	
Chemical/metal[a]	A	B	C	D	F	G	H	I	J	K	L	M	N	O
Aniline		14,000	550,000											
Benzyl Chloride	3,000													
Bis-(2-ethyl-hexyl)-phthalate		500		<100							200	230	<10	<10
Butylbenzyl-phthalate				<100	120	450	<5	8	160	140				
Chlordane											<60	<60	19,000	19,000
Chlorophenylisocyanate		21,000												
Cresol(s)									2,000	2,500				
Diethylphthalate					620	1,300	240	240						
m-Dichlorobenzene		23,000												
o-Dichlorobenzene		46,000												
p-Dichlorobenzene		59,000												
m-Dinitrobenzene			<100											
Diphenylamine			6,200											
2,4-Dimethylphenol									500	2,000				
Hexachlorobutadiene											<10	<10	<10	<10
Hexachloroethane	560													
Hexachlorocyclo-pentadiene											<10	<10	230	260
Isophorone				<100										
Mononitrobenzene			<100											
Naphthalene	<100			<100	250	450	44	49	450	490	38	33	<10	<10
Phenol				34,000	1,500	1,700	1,800	2,900						
Phenylenediamine			2,300											

Phenylisocyanate		160,000												
Trans-1,4-dichloro-2-butene	59,000													
1,2,4-Trichlorobenzene		290												
Benzene				<3							46,000	58,000	<10	<10
Carbon Tetrachloride	68,000	44,000		<2	3,700	4,400	<10	<10	5,900	5,700	9,100	11,300	<10	<10
Chlorobenzene		4,100									390	500	<10	<10
Chloromethane		1,200												
Chloroform	2,900				170	270	21	22			110	60	<10	<10
Cis-1,4-dichloro-2-butene	18,000													
Methylene Bromide											<10	<10	3,100	4,700
Methylene Chloride	21,000										100	340	44	60
Methyl Ethyl Ketone				27					18,000	38,000				
Tetrachloroethylene	11,000			<1	7,100	9,800	1,600	1,300	7,900	8,100	87	150	<10	<10
Toluene	240,000			110	32,000	45,300	2,900	2,700	56,000	48,000	160,000	390,000	22	20
1,1,1-Trichloroethane	<100								24,000	16,000	330	230	<10	<10
Trichloroethylene	4,000	40,000		<1	3,700	4,400	90	85	8,100	7,800	8,300	10,300	<10	<10
Ag	<3				<1	<1	<1	<1			<1	<1		
As	<24				<20	<20	<20	<20	<14	<14	<23	<23		
Ba	<7				110	140	6	7	1,160	1,150	990	1,100		
Be	<2				<1	<1	<1	<1	<1	<1	<1	<1		
Cd	<5				6	6	<1	<1	153	15	49	55		
Cr	<5				50	57	3	3	431	425	250	290		
Hg	<22				<10	<10	<10	<10	<4	<4	<50	<50		
Ni	<68				7	7	2	2	26	27	<4	<4		
Pb	<19				140	150	<10	11	1,830	1,800	1,200	1,300		
Sb	<12				58	61	<10	<10	437	373	<24	<24		
Se	<470				340	<100	<100	<100	<21	<21	<160	<160		
TL	<23				<20	<20	<20	<20	<9	<9	<22	<22		
Si									<1	<1				
pH		2		8	7	7	6	7				7	7	7
Water (%)	2		5	94	38	48	95	67	21	23	5	5	94	96

[a]Data from US EPA [1984]. Waste samples B, F, G, J, K, L, and M were spiked with carbon tetrachloride and trichloroethylene; therefore, the concentrations of these two chemicals in these wastes reflect this addition.

Positive Controls

In the presence of S9, the positive control was 2-aminoanthracene (2-AA; Sigma, St. Louis) at a starting concentration of 5 μg/ml in the first well. In the absence of S9, the positive control was 2-nitrofluorene (2-NF; Sigma) at a starting concentration of 300 μg/ml in the first well. Both compounds were dissolved in acetone (Fisher Scientific) immediately prior to use.

Microsuspension Assay

Plastic, sterile 96-well microtiter plates (Corning Laboratory Sciences Co.) were used for the microsuspension assay. The first well in a dilution series received 250 μl of supplemented minimal medium and 50 μl of either the crude waste sample or the positive or negative (medium) control. Successive wells in the series received 150 μl of the medium, and twofold serial dilutions of the wastes and controls were then performed by transferring 150 μl from well to well. Dilutions were carried routinely into consecutive plates to noncytotoxic doses in order to assure the identification of the dose range. Wastes N and O, which were neither cytotoxic nor genotoxic at the highest concentration tested (10% vol/vol based on the above protocol), were diluted to a higher starting concentration in supplemented minimal medium, and 300 μl of this dilution was deposited into the first well. Serial dilutions were then performed as described above.

Following dilution of the wastes, wells were inoculated with 75 μl ($\sim 2 \times 10^6$ cells) of the resuspended log-phase culture of $WP2_s(\lambda)$ and 25 μl of medium or S9 mix, prepared as described by Houk and DeMarini [1987] using rat liver homogenate (23.63 mg protein per ml S9) from male Sprague-Dawley rats. The contents of the microtiter plate were mixed using a Minimixer (Fisher Scientific), and the plates were enclosed in individual platic zip-lock bags to reduce evaporation and to trap volatile compounds. Plates were incubated overnight (20 hr) at 37°C. Following incubation, wells were scored for turbidity (cell growth) or clarity (cytotoxicity and/or inhibition of cell growth).

Assay for Plaque Formation

The method of Rossman et al [1984] was performed with modifications [Houk and DeMarini, 1987]. An aliquot of 100 μl from selected wells of the microtiter plate was diluted in 9.9 ml of supplemented minimal medium, and chloroform (10 μl/ml) was added to kill viable lysogens. To ~2.5 ml of molten top agar (0.65% Bacto agar and 10 mM NaCl) were added 500 μl of the diluted phage and 200 μl of the overnight culture of SR714. Contents of the tubes were vortexed and poured onto bottom agar made of half-strength tryptone medium [Arber et al, 1983]. Plates were incubated overnight at 35°C, after which plaques were counted by hand. Dilution tubes were sampled in duplicate, and all experiments were performed at least twice.

RESULTS

The cytotoxic responses of the waste samples are noted in Table III. All of the wastes elicited some form of cytotoxic response manifested in one of three ways:

1) as clear, or nonturbid, wells (eg, wastes A and B);

2) as partially turbid wells yielding few or no plaque-forming units (PFU) per plate (eg, wastes J and K);

3) as fully turbid wells yielding a reduced number of PFU/plate compared to the medium controls (eg, wastes F, N, and O).

Consistent with previous observations [Houk and DeMarini, 1987; Rossman et al, 1984, 1985], no or few phage were isolated from clear wells, with the exception of waste B in the presence of S9 and G in the absence of S9 (Table III). Because these waste samples are complex mixtures, it is possible that some cytotoxic compounds in these samples exerted their cytotoxic effects after genotoxic compounds had already begun inducing significant levels of prophage.

The background, or spontaneous, PFU/plate were calculated by averaging results from eight medium control wells (with and without S9) for each experiment. All of the background values were then used to calculate the upper limit of the 99% confidence interval in the presence and absence of S9. In the presence of S9, the upper limit of the 99% confidence interval was 102 PFU/plate; in the absence of S9 it was 30 PFU/plate. Our criterion for a positive response was an induced PFU/plate (the observed minus the background) that reached or exceeded these confidence intervals.

The response for each waste sample based on this criterion is shown in Table III. Replicate experiments were conducted to confirm each finding, and raw data from a representative experiment for each of the wastes are presented. Selected doses illustrate the response range for each waste tested. In addition to showing the induced PFU/plate (observed minus background), Table III also indicates the fold increase over background. For most of the positive waste samples, the dose that produced either 102 induced PFU/plate (+S9) or 30 induced PFU/plate (−S9) generally resulted in a three- or fourfold increase over the background.

Eleven of the 14 crude hazardous waste samples induced prophage. Only two of the wastes (D and J) required S9 for induction, and none of the waste samples were genotoxic only in the absence of S9. S9 appeared to reduce the genotoxic potencies of wastes A, B, L, F, and M, but it enhanced the potencies of wastes H and I (Table IV).

Dose-response curves for the positive waste samples are illustrated in Figures 1–3. Although wastes A, C, and G were positive in the presence of S9 (Table III), they did not produce the simple, linear dose responses exhibited by the other waste samples. Instead, wastes A and C produced reproducible, multimodal dose-response curves that ranged over 4 orders of magnitude (Fig. 3), and waste G demonstrated a threshold response (data not plotted).

Using the linear portions of the dose-response curves in Figures 1 and 2, we calculated the concentration required by each waste to produce an induced PFU/plate equal to the upper limits of the 99% confidence intervals of the medium controls (the minimum concentration of the waste required to produce a positive response based on our criterion described previously). The waste samples were then ranked from the most to the least potent (Table IV). The petrochemical and pharmaceutical wastes (samples A and B) were the most potent (Fig. 2; Table IV). However, the potencies of most of the remaining wastes did not appear to be significantly different from one another.

TABLE III. Induction of Prophage Lambda by Hazardous Wastes[a]

	+S9				−S9			
Waste	Dose (μg/ml)	PFU per plate[a]	Induced PFU per plate[b]	Fold increase	Dose (μg/ml)	PFU per plate	Induced PFU per plate	Fold increase
A	0	21	0	—	0	12	0	—
	5.0×10^{-5}	82	61	3.9	2.0×10^{-7}	30	18	2.5
	2.0×10^{-4}	160	139	7.6	4.0×10^{-7}	49	37	4.1
	1.2×10^{-2}	320	299	15.2	8.0×10^{-7}	60	48	5.0
	2.5×10^{-2}	388	367	18.5	1.5×10^{-6}	2(T)	—	—
	1.5	552	531	26.3				
	50	241	220	11.5				
	200	182	161	8.7				
	400	0(T)	—	—				
Response		+				+		
B	0	21	0	—	0	12	0	—
	0.05	249	228	11.9	2.0×10^{-7}	24(P)	12	2.0
	0.1	421	400	20.0	4.0×10^{-7}	49(P)	37	4.1
	0.2	582	561	27.2	8.0×10^{-7}	106(P)	94	8.8
	0.4	957	936	45.6	1.5×10^{-6}	136(P)	124	11.8
	0.8	561(T,P)	540	26.7	3.0×10^{-6}	572(t,P)	560	47.8
	1.6	2(T,P)	—	—	6.0×10^{-6}	24(P)	12	2.0
Response		+				+		
C	0	21	0	—	0	26	0	—
	1.0×10^{-2}	29	8	1.4	200	24	—	—
	5.0×10^{-2}	56	35	2.7	400	30	4	1.2
	0.2	125	104	6.0	800	57	31	2.2
	0.8	81	60	3.9	1,600	68	42	2.6
	3	53	32	2.5	3,100	0(T)	—	—
	12	48	27	2.3	6,300	0(T)	—	—
	50	136	115	6.5				
	200	9(t)	—	—				
Response		+				+		

D	0	52		0	—	0	12	0	—
	800	76		24	1.5	800	19	7	1.6
	1,600	138		86	2.7	1,600	12	0	1.0
	3,100	131		79	2.5	3,100	12	0	1.0
	6,300	148		96	2.8	6,300	11	—	—
	12,500	218		166	4.2	12,500	12	0	1.0
	25,000	23(t)		—	—	25,000	0(T)	—	—
Response			+					−	
F	0	53		0	—	0	9	0	—
	100	63		10	1.2	200	15	4	1.7
	200	78		25	1.5	800	33	24	3.7
	400	103		50	1.9	1,600	75	66	8.3
	3,100	115		62	2.2	3,100	235	226	26.1
	12,500	259		206	4.9	6,300	721	712	80.1
	25,000	3,755(P)		3,702	70.8	12,500	1,960	1,951	217.8
	50,000	416(P)		363	7.8	25,000	962	953	106.9
	100,000	0(P)		—	—	50,000	1(P)	—	—
Response			+					+	
G	0	89		0	—	0	19	0	—
	1,250	353		264	4.0	780	20	1	1.0
	2,500	346		257	3.9	3,100	100(t)	81	5.3
	5,000	402		313	4.5	5,000	950(t)	931	50.0
	6,300	400		311	4.5	6,250	5,430(t)	5,411	285.6
	12,500	466		377	5.2	10,000	3,450(t)	3,431	181.6
	25,000	4,200(P)		4,111	47.2	20,000	2,300(T)	2,281	121.1
	40,000	1,800(P)		1,711	20.2	25,000	71(T,P)	52	3.7
	50,000	17(P)		—	—	50,000	0(T,P)	—	—
Response			+					+	
H	0	53		0	—	0	9	0	—
	400	33		—	—	400	10	1	1.1
	800	76		23	1.4	800	14	5	1.6
	1,600	202		149	3.8	1,600	19	10	2.1
	3,100	373		320	7.0	3,100	42	33	4.7
	6,300	591		538	11.2	6,300	64	55	7.1
	12,500	792		739	14.9	12,500	138	129	15.3
	25,000	0(P)		—	—	25,000	0(P)	—	—
Response			+					+	

(continued)

TABLE III. Induction of Prophage Lambda by Hazardous Wastes* (Continued)

	+S9				−S9			
Waste	Dose (μg/ml)	PFU per plate[a]	Induced PFU per plate[b]	Fold increase	Dose (μg/ml)	PFU per plate	Induced PFU per plate	Fold increase
I	0	53	0	—	0	9	0	—
	800	88	35	1.7	800	11	2	1.2
	1,600	361	308	6.8	1,600	34	25	3.8
	3,100	768	715	14.5	3,100	68	59	7.6
	6,300	740	687	14.0	6,300	44	35	4.9
	12,500	936	883	17.7	12,500	27	18	3.0
	25,000	0(P)	—	—	25,000	0(P)	—	—
Response		+				+		
J	0	62	0	—	0	12	0	—
	12	56	—	—	6	10	—	—
	50	76	14	1.2	12	13	1	1.1
	100	111	49	1.8	25	15	3	1.3
	200	173	111	2.8	50	26	14	2.2
	400	133	71	2.1	100	28(t)	16	2.3
	800	12(t)	—	—	200	10(t)	—	—
Response		+				−		
K	0	62	0	—	0	26	0	—
	50	69	7	1.1	200	27	1	1.0
	100	60	—	—	400	29	3	1.1
	200	66	4	1.1	800	3(t)	—	—
	400	87	25	1.4	1,600	3(t)	—	—
	800	16(t)	—	—	3,100	1(t)	—	—
Response		−				−		
L	0	62	0	—	0	12	0	—
	12	69	7	1.1	800	29	17	2.4
	400	116	54	1.9	1,600	69	57	5.8
	6,300	204	142	3.3	3,100	101	89	8.4
	12,500	464(F)	402	7.5	6,300	266	254	22.2
	25,000	2,350(F)	2,288	37.9	12,500	1,692	1,680	141.0
	50,000	0(F)	—	—	25,000	5(F)	—	—
Response		+				+		

(continued)

M	0	62	0	—	0	12	0	—
	3,100	132	70	2.1	1,600	53	41	4.4
	6,300	131	69	2.1	3,100	79	67	6.6
	12,500	184(F)	122	3.0	6,300	224	212	18.7
	25,000	406(F)	344	6.5	12,500	2,085	2,073	173.8
	50,000	287(F)	225	4.6	25,000	7,590(F)	7,578	632.6
	100,000	0(F)	—	—	50,000	0(F)	—	—
Response		+				+		
N	0	87	0	—	0	26	0	—
	37,500	107	20	1.2	37,500	28	2	1.1
	50,000	139	52	1.6	50,000	23	—	—
	75,000	135	48	1.6	75,000	23	—	—
	100,000	142	55	1.6	100,000	24	—	—
	200,000	23	—	—	200,000	16	—	—
	300,000	18	—	—	300,000	10	—	—
Response		−				−		
O	0	87	0	—	0	26	0	—
	37,500	77	—	—	37,500	12	—	—
	50,000	84	—	—	50,000	11	—	—
	75,000	94	7	1.1	75,000	12	—	—
	100,000	100	13	1.1	100,000	16	—	—
	200,000	31	—	—	200,000	10	—	—
	300,000	18	—	—	300,000	6	—	—
Response		−				−		

*The positive control results were 2-aminoanthracene (0.3 μg/ml) 716 ± 288 PFU/plate; 2-nitrofluorene (150 μg/ml) 400 ± 264 PFU/plate (average and standard deviation of six experiments).

[a]Each value is the average of two plates from a representative experiment. T, toxic (clear well); t, slightly toxic (slightly turbid well); P, precipitate; F, oily film on surface of medium.

[b]Induced PFU/plate = observed minus background.

TABLE IV. Relative Potencies of Wastes

Waste	Concentration (μg/ml) required to produce an induced PFU/plate equal to the upper limit of the 99% confidence interval based on the medium controls[a]			
	+S9	Rank	−S9	Rank
B	0.03	(1)	3×10^{-7}	(1)
A	+[b]		4×10^{-7}	(2)
C	+		1,082	(3)
J	185	(2)	−	
I	976	(3)	1,888	(7)
H	1,346	(4)	3,355	(8)
L	3,029	(5)	1,200	(4)
D	6,076	(6)	−	
F	6,444	(7)	1,490	(5)
M	9,561	(8)	1,638	(6)
G	+		3,456	(9)

[a]This is the concentration (μg/ml) required to produce 102 induced PFU/plate (+S9) or 30 induced PFU/plate (−S9).
[b]Potency value incalculable because of nonlinear dose response (see text).

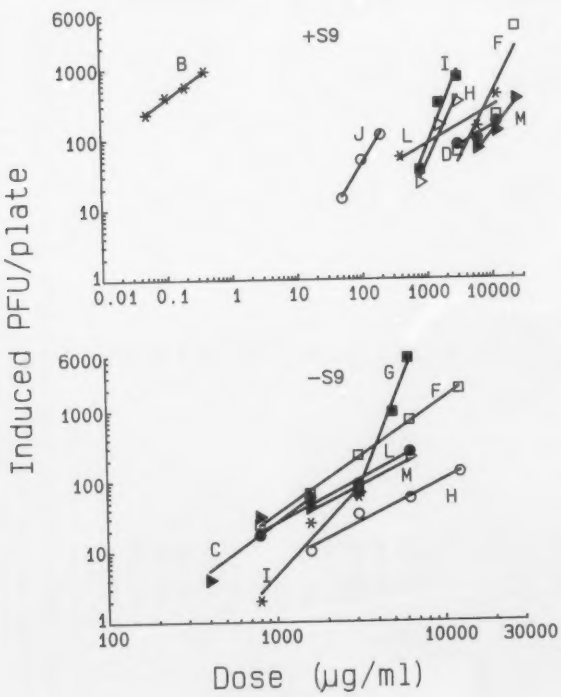

Fig. 1. Dose-response curves of prophage induction by genotoxic hazardous waste samples in the presence and absence of S9. Only the linear portions of the dose-response curves are shown, and the correlation coefficients ("r^2" values) are 0.99 for all of the curves except L (0.97) and F (0.92) in the presence of S9 and C (0.91), M (0.98), and G (0.88) in the absence of S9.

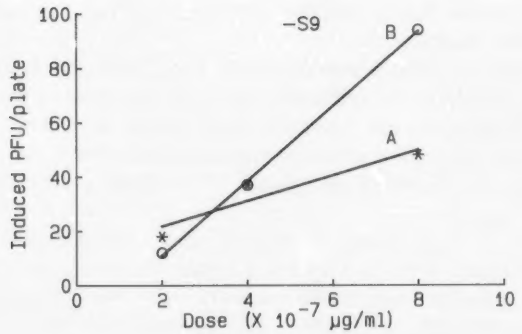

Fig. 2. Linear portions of the dose-response curves of prophage induction by hazardous waste samples A and B in the absence of S9. Correlation coefficients are 0.94 and 0.99 for A and B, respectively.

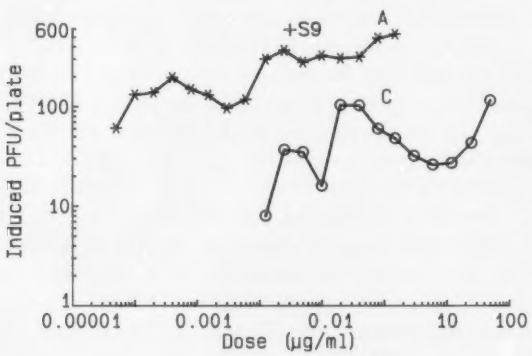

Fig. 3. Dose-response curves for waste samples A and C in the presence of S9.

DISCUSSION

The selection of wastes used in this study was based solely on the availability of a diverse set of waste samples that had been at least partially chemically characterized. Thus, these samples do not in any way reflect the universe of hazardous wastes nor should they be viewed as representative of wastes from similar industries or processes. The partial chemical characterization available for these wastes (Table II) is more extensive than would ordinarily be available for waste samples, many of which might be of completely unknown chemical composition. However, even this level of chemical analysis is inadequate to distinguish a genotoxic from a nongenotoxic waste or to indicate which waste might be more genotoxic than another.

For example, the chemical analysis of the petrochemical and pharmaceutical waste samples (A and B, Table II) does not necessarily suggest the remarkable cytotoxic and genotoxic potencies exhibited by these two samples compared to the other waste samples (Tables III, IV). Likewise, the finding that waste samples K, N, and O were not genotoxic is not readily apparent from their chemical profiles, which are relatively indistinguishable from those of the genotoxic waste samples (Table II). It also should be noted that waste sample K, one of the wastes spiked with carbon tetrachloride and trichloroethylene (Table II), was negative both in the presence and

absence of S9, suggesting that the addition of these compounds did not interfere with the assessment of the wastes.

Hazardous waste samples are so physically and chemically diverse that routine test protocols may not always be applicable. For example, most of the waste samples tested here were highly toxic and contained toxic volatile compounds that interfered with induction and/or survival of prophage from adjacent rows of the same microtiter dish (in some cases by inhibiting the growth of lysogenic cells). This necessitated evaluating each of the wastes in separate microtiter dishes and, additionally, placing doses in nonadjacent wells. In a majority of cases, complex mixtures must be extracted or fractionated prior to bioassay to make them compatible with the test system. However, with the Microscreen assay we were able to test the crude wastes directly, saving considerable time, effort, and expense. The unfractionated wastes posed no sterility problems, probably because of their extreme toxicity. Other wastes, however, may contain microbial contaminants that would necessitate the preparation of organic extracts, possibly involving filtration of the extracts. Finally, although the color of complex mixtures has been shown to complicate data analysis for some SOS assays that rely on colorimetric measurements [Whong et al, 1986], the dark color of these wastes did not interfere with the interpretation of results in this assay.

Of the 20 genotoxic responses observed in the present study (Table III), only three were nonlinear. All three occurred in the presence of S9. Wastes A and C yielded multimodal dose-response curves (Fig. 3), and waste G showed a threshold response (Table III, data not plotted). These nonlinear responses may have been due to different compounds (or metabolites) in the mixture exerting their genotoxic effect at different doses. Given the complex chemical makeup of these hazardous waste samples, the incidence of simple, monotonically increasing dose responses seemed surprising. However, linear dose responses also have been observed with the vast majority of complex mixtures tested with *Salmonella*, including the hazardous wastes tested here [DeMarini et al, 1987b].

With the exception of waste samples A and B, the potency values of the wastes were similar (Table IV). This may have been due to the fact that most of these wastes were composited from a variety of sources. Thus, mixtures of potent and weak wastes may have resulted in samples with similar average potencies. The wastes that did demonstrate vastly different potencies from the rest (samples A and B) were not composite wastes but were from two distinct industrial sources, the petrochemical and pharmaceutical industries. Based on the similarity of the potency values (Table IV) and the clustering of the dose-response curves of the composite wastes (Fig. 1), we place little significance in the potency rankings of these wastes and view the potencies as indistinguishable from one another.

Table V compares the mutagenicity of these waste samples in *Salmonella* to the ability of these waste samples to induce prophage. All but one of the wastes that were mutagenic in *Salmonella* also induced prophage. This is consistent with studies of pure compounds, which show that for those compounds tested, most chemicals that are mutagenic in *Salmonella* also induce prophage and/or the SOS response [Elespuru, 1984; Oda et al, 1985; Ohta et al, 1984; Quillardet et al, 1985; Rossman et al, 1986]. Thus, at least some concordance between the assays is not surprising. The inability to detect waste sample O in the phage-induction assay may have been due to the toxic or inhibitory effect of some component(s) of the mixture, as evidenced by a reduction in PFU/plate at concentrations above 100,000 μg/ml (Table III).

TABLE V. Comparison of Prophage Induction in *Escherichia coli* and Mutagenesis in *Salmonella typhimurium*

Waste	Prophage induction in *E. coli*	Mutagenicity in *S. typhimurium*[a]
A	+	+
B	+	–
C	+	+
D	+	–
F	+	+
G	+	+
H	+	–
I	+	–
J	+	–
K	–	–
L	+	+
M	+	+
N	–	–
O	–	+

[a]From DeMarini et al [1987b]. Wastes were tested in strains TA98 and TA100. A positive response in either strain, with or without S9, was sufficient for a positive summary response reported here.

The phage-induction assay detected five waste samples that were not mutagenic in *Salmonella* (Table V). As mentioned previously, the Microscreen phage-induction assay has been shown to detect some carcinogenic metals [Rossman et al, 1984], chlorinated organics [Houk and DeMarini, 1987], and solvents [Houk and DeMarini, in prep.] that are not mutagenic in *Salmonella* [Kier et al, 1986; Zieger, 1987; Zeiger and Tennant, 1986]. Metals and compounds of these types are present in most of the waste samples studied here (Table II), and the ability of some of these compounds to induce prophage may account for the detection by the phage-induction assay of the five additional waste samples that were not detected by the *Salmonella* assay. Furthermore, accumulating evidence indicates that phage induction (and the SOS response in general) is a broader genetic endpoint than reverse mutation in bacteria; it occurs by a variety of mechanisms [Elespuru, 1984, 1987; Hofnung and Quillardet, 1986] and involves a number of classes of genetic damage, including alkylations, intercalations, cross-links, strand scissions, and oxidative deaminations [Rossman et al, 1985; Elespuru, 1984]. This, too, could account in part for the difference in detection sensitivities.

The ability of hazardous wastes to produce biological effects other than genotoxicity should be explored. In addition to prophage induction and mutagenicity in *Salmonella* [DeMarini et al, 1987b], 11 of the wastes studied here have also been examined for their lethality and hepatic toxicity in rats [Simmons et al, in prep.] because of the presence of known hepatotoxins in these waste samples (Table II). The results show that most of the wastes were potent hepatotoxins—even more so than suggested by the chemical characterizations of the wastes.

The limitations of chemical analyses for predicting the biological effects of these wastes argue in favor of bioassays to help characterize the possible hazardous nature of wastes. In combination with chemical analysis, assessments of the biological effects

of wastes could contribute valuable information regarding the hazardous nature and risk associated with exposure to industrial wastes and effluents. Our data suggest that the Microscreen phage-induction assay may provide a simple, sensitive, and inexpensive way to screen hazardous wastes for an important biological endpoint, genotoxicity.

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